Morphological diversity of the invasive plant lesser celandine (*Ranunculus ficaria*)

Undergraduate Research Thesis

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Abstract

Lesser celandine (*Ranunculus ficaria*) is a spring ephemeral wildflower native to Europe and introduced in the eastern United States as a garden flower. The species escaped cultivation and invaded riparian woodlands and floodplains. Although genetic and morphological diversity of *R. ficaria* has been studied in Europe, few studies have examined morphological traits in North America under controlled conditions. This experiment aims to document leaf, floral, and reproductive trait diversity in populations of *R. ficaria* in four cities: Louisville, KY, and Cincinnati, Columbus, and Cleveland, OH. I collected a total of 129 living plant samples from 43 sites along riverways. I replanted samples and measured trait values in an outdoor common garden experiment in Spring 2020. I found a high prevalence of clonal reproductive structures in all four cities and all riverways. The presence of clonal reproductive structures was correlated with the number of leaves. Flowers were produced by plants collected from 31 of 43 sites, but plants from Cincinnati did not flower. Surprisingly, all 31 sites which produced flowers also produced enlarged achenes, indicating possible seed production. I found that plants from Cleveland have smaller flowers and longer leaves than plants from the other cities. Plants from east Columbus riverways have larger flowers than plants from central Columbus riverways. In summary, I documented both between-city and within-city morphological diversity.

Introduction

Clonal growth and reproduction are present in many angiosperm lineages, complementing the ability to reproduce sexually (Barrett 2015). There are numerous advantages to clonal reproduction. One advantage is that it enables a successful genotype (a 'genet') to produce many 'ramets' which can colonize an area quickly (Barrett 2015). However, a species which predominately reproduces clonally is susceptible to multiple genetic effects which might reduce fitness. The first is a reduction in genetic diversity. For example, the Cavendish banana (*Musa spp*.) variety is commercially propagated clonally, making the plants almost genetically identical and thus almost equally susceptible to a Fusiarium wilt race (Huang and Ko 2004). The second negative genetic effect is the gradual buildup of deleterious mutations in a clonal line (Barrett 2015). For example, older clones of quaking aspen (*Populus tremuloides*) in Western North America tend to have more sterile pollen, a finding attributed to deleterious mutations (Barrett 2015).

Introducing genetic diversity into a population can increase the fitness of the members. This is due to the introduction of novel adaptive alleles or the increase in heterozygosity at individual loci (Lavergne and Molofsky 2007). For example, reed canarygrass (*Phalaris arundinacea*), an invasive species, was introduced multiple times into North America from different places in Europe. The North American populations of *P. arundinacea* tend to have a higher diversity of alleles at each locus than the European populations. The North American populations have greater broad-sense heritability for multiple traits and greater phenotypic plasticity along a moisture gradient (Lavergne and Molofsky 2007). Multiple introductions of the same invasive species from different regions, given that the species can reproduce sexually, can introduce more variation for natural selection to act on.

Lesser celandine (*Ranunculus ficaria*) is a species of spring ephemeral wildflower that is native to Europe and Northern Africa (Axtell et. al. 2010). It was introduced to North America, New Zealand, and Japan as a horticultural plant (Axtell et. al. 2010, iNaturalist 2021). In North America, *R. ficaria* is

naturalized and invasive in riparian and floodplain habitats in much of the eastern United States from Alabama into Canada, as well as in the Pacific Northwest (Axtell et. al. 2010). It begins producing leaves in late winter, flowers in spring, and senesces before the middle of summer (Axtell et. al. 2010).

There are five subspecies of *R. ficaria*: subspecies *calthifolius, ficaria, bulbifer, ficariiformis*, and *chrysocephalus* (Sell 1994). In Europe, the subspecies have mostly overlapping ranges, and are also transported to different regions for cultivation as a garden plant (Sell 1994). All five of the European subspecies are found in North America (Post et. al. 2009). The biggest difference between subspecies is their reproductive biology. Two of the subspecies, *bulbifer* and *ficariiformis*, produce specialized structures called axillary bulbils for clonal reproduction. These axillary bulbils are ellipsoid or spherical structures, about 0.5-1 cm in diameter, which grow from leaf axils just before plant senescence. The bulbils can be spread along waterways during flooding events or tracked to new areas by animals or people (Sell 1994, Axtell et. al. 2010). All five subspecies produce flowers and belowground tubers (Axtell et. al. 2010). It has been reported, from the United Kingdom, that subspecies *bulbifer* only rarely produces viable seeds and thus relies on clonal reproduction (Metcalfe 1939). Interestingly, the five subspecies also differ in ploidy. Both subspecies that produce axillary bulbils, along with one that does not, *chrysocephalus*, are tetraploid (or occasionally triploid). The remaining two, *calthifolius* and *ficaria*, are diploid.

Apart from reproductive biology, the subspecies of *R. ficaria* can be separated based on the dimensions of flowers and leaves. Subspecies, when studied in Europe, varied in maximum leaf petiole lengths, leaf blade dimensions, flower widths, achene dimensions, and crowding of leaves into a rosette vs. elongation of stems (Sell 1994). Post and colleagues measured morphological traits of North American *R. ficaria* from herbarium specimens. They found significant differences between subspecies in leaf length, leaf width, petiole length, petal length, petal width, achene length, and achene width

(Post et. al. 2009). However, subspecies are similar enough to one another that overlap occurs in trait value distributions (Post et. al. 2009).

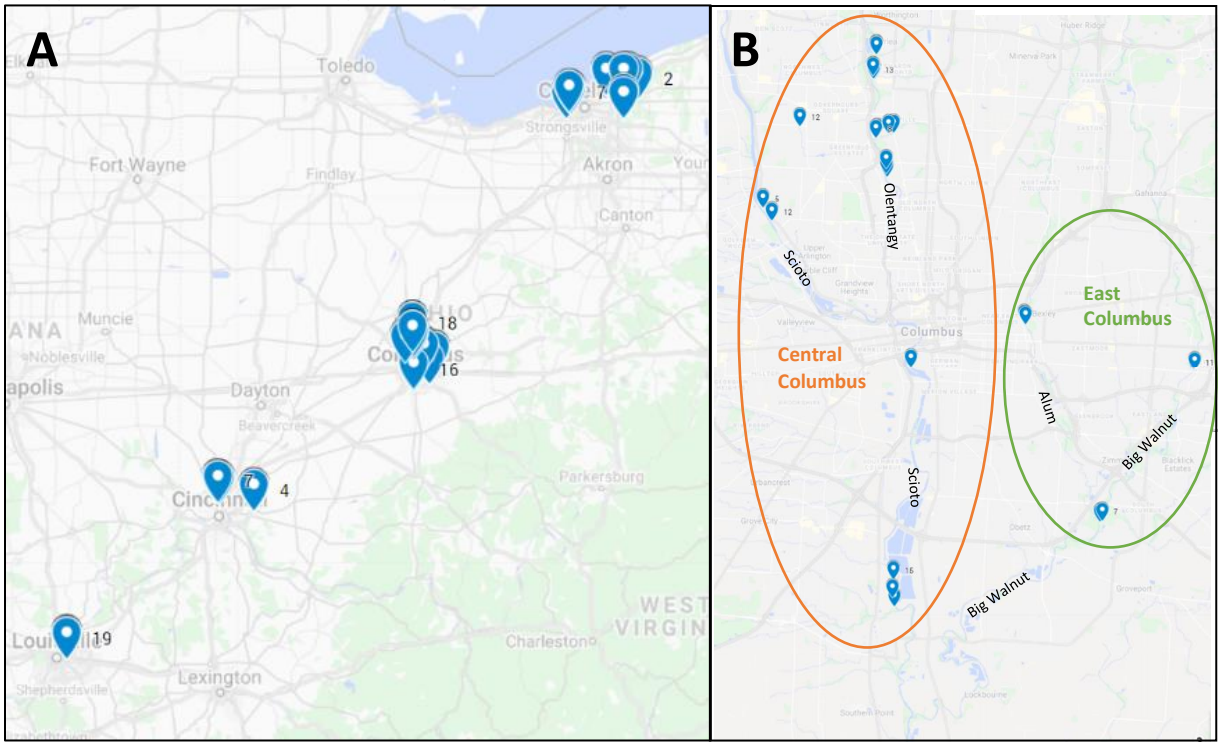
Purpose

We have done previous research on the genetic diversity of lesser celandine in Ohio (Mattingly, Tayal, Rauschert and Hovick; manuscript in preparation). We used primer-based techniques to genotype *R. ficaria* samples collected from three Ohio cities. Preliminary results identified both within- and between-city genetic diversity. Considering the genetic data, I designed this experiment to document morphological trait variation under common garden conditions. By cultivating plants under identical environmental conditions, I hope to reveal morphological variation attributable to genetics. Specifically, I aim to measure traits related to subspecies identification and reproductive biology.

Methods

We visited a total of 43 GPS-marked collection sites in four Midwest cities between February 16th, 2020 and March 9th, 2020 (Figure 1). During this time, celandine plants are growing leaves to begin photosynthesizing while canopy trees are still bare. At each GPS site, we selected three locations greater than 15 feet apart from each other (if possible). At each of the three locations, we collected a clump of celandine plants. We took care to dig underneath the long tubers of the celandine. Thus, we stored three ziplock bags of samples for each GPS-marked collection site (and so the total number of samples we collected was 43*3 = 129). We sealed the bags and stored them at ~4 °C until replanting.

Figure 1. A) Sampling sites in Louisville (2 sites), Cincinnati (5 sites), Columbus (27 sites), and Cleveland (9 sites). B) Closeup of Columbus, OH, showing riverways and the distinction between East Columbus (6 sites) and Central Columbus (21 sites).



I replanted all samples between March 22nd, 2020 and March 28th, 2020. For each bag, I washed away the soil surrounding the celandine roots by agitating the soil with a water sprayer and shaking under water. I selected three plants from the bag based on lack of major damage. For the plant with the longest tuber, I recorded maximum tuber length and number of tubers. I prepared a double-labeled 4-inch pot filled with moist potting soil (Lambert LM-3 All Purpose Mix). I potted all three selected plants from a bag into a single pot, with the plant designated for data collection being placed nearest to the label (the other two plants were potted in case the first died). After I had repeated the process for the 129 samples, I randomized the order of the pots and placed them into greenhouse trays with holes underneath. I placed the trays in an outdoor location in Columbus, Ohio with sun for ~1/3 of the day and shade for ~2/3 of the day.

I monitored the plants for flowering and watered as needed. I collected data on the first flower that opened for each pot (for the 59 plants which flowered; see Table 1 and Figure 2 for details). I

recorded a suite of floral traits using digital calipers. In order to eventually measure seed production, I used a tie to mark each flower from which I collected data. I collected all floral data between April 1st and April 28th.

Between April 20th and April 23rd, I collected leaf data for the first plant in every pot (Table 1, Figure 2). I measured a suite of traits for the single leaf with the longest blade length. For the first fifty samples, I painted a (0.5 cm)*(0.5 cm) area of clear nail polish onto the abaxial side of the lobe of the leaf. After 0.5-1 hour, I peeled off the dried nail polish with cellophane tape, creating a stomata impression. I placed the cellophane tape pieces onto glass slides and visualized them under a light microscope at 400x (40x objective * 10x eyepiece). I photographed three different locations on each piece of cellophane tape and each photograph contained up to six stomata. I measured stomata length in pixels using ImageJ software (Rasband 2018). I averaged all stomata lengths for a given leaf peel into a single variable, average_stomata_length_pixels.

I collected data on seed and bulbil production between May 17th and May 21st (Table 1). During this time, the celandine plants began to senesce as they entered summer dormancy. I recorded how many of the achenes in each flower became expanded. I recorded the presence or absence of bulbils in the leaf axils.

Figure 2. Diagram of selected traits collected from plants. Some traits are omitted.

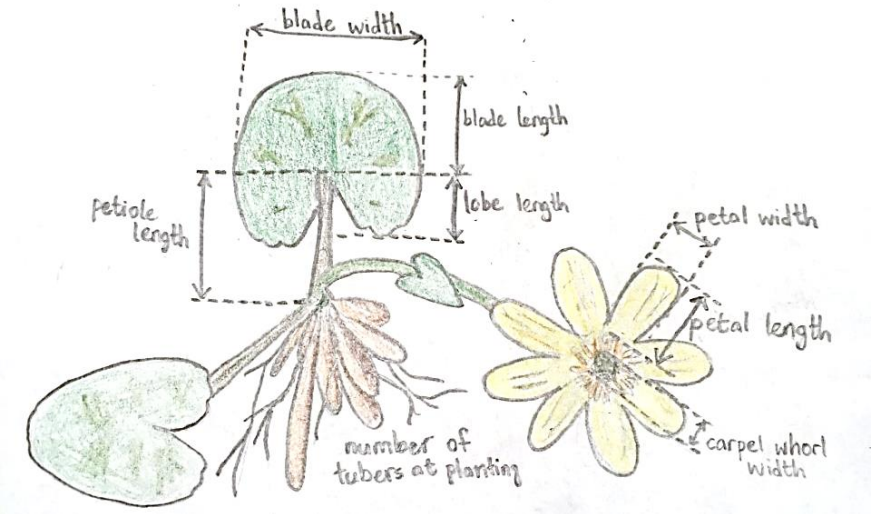


Table 1. Important morphological traits recorded for celandine plants.

Trait name	Category	Unit	Trait Explanation
num_tubers	Below ground		Number of tubers on plant 1 in the pot during planting
length_largest_tuber	Below ground	mm	Length of longest tuber on plant 1, measured at planting
pedicel	Flower	mm	Distance from base of sepals down to base of the uppermost leaf
distal_internode	Flower	mm	Distance from uppermost leaf base down to the next-highest leaf base
num_sepals	Flower		The number of sepals
num_petals	Flower		The number of petals
sepal_length	Flower	mm	Length from base of sepal to tip of sepal
petal_length	Flower	mm	Length from base of the petal to the petal tip
petal_width	Flower	mm	Width at widest point of a flattened petal
carpel_whorl_width	Flower	mm	Diameter of the spherical carpel whorl
num_leaves	Leaf		The number of leaves of plant 1 in the pot at the time of leaf data collection
blade_length	Leaf	mm	Distance from the point where petiole meets blade to the blade tip
lobe_length	Leaf	mm	Distance from the tip of the lobe to the point where petiole meets blade
blade_width	Leaf	mm	Width of the blade
petiole_length	Leaf	mm	Distance from petiole base to the point where petiole meets blade
num_viable_achenes	Achene		Number of achenes that became enlarged (if the achenes already fell off then the number was inferred)
max_achene_length	Achene	mm	Maximum achene length of the achenes that are enlarged
axillary_bulbils_present	Bulbil		Presence of bulbils in leaf axils - either in axils in a basal rosette or in axils higher up on a stem
average_stomata_length_pixels	Leaf	pixels (at 400x)	The average length in pixels over all the stomata measured for a particular leaf peel

I investigated whether plants with more tubers during planting were more likely to produce axillary bulbils. I also asked whether the number of leaves on a plant was related to axillary bulbil production. I used a one-sided Mann-Whitney test (a Wilcoxon rank sum test) with continuity correction (Miller and Miller 2014).

Before running other analyses, I calculated Pearson's correlation coefficient for each combination of continuous traits in my dataset. Because blade width and blade length were highly correlated (r=0.874), I combined the two traits into a single variable by taking their ratio. No other pairs of traits had correlations higher than r=0.8.

To reduce the dimensionality of the data and enable visualization, I ran principal components analysis (PCA) on a selection of continuous traits. The first PCA incorporated both a size-covariate (number of tubers at planting) as well as all continuous leaf trait data (blade length divided by width, lobe length, and petiole length). In the second PCA, I added floral data at the cost of reducing sample size from 129 to 59. I included all the variables from the first PCA, as well as sepal length, petal length, petal width, and carpel whorl width. In both PCAs, I transformed the variables to give each a mean of zero and a standard deviation of one. I implemented PCA with the prcomp function in R version 4.0.2 (R Foundation for Statistical Computing 2020).

To analyze morphological diversity by region of collection, I divided the data into five groups: Louisville, KY, Cincinnati, OH, Cleveland, OH, eastern Columbus, OH, and central Columbus, OH. A distinction was made between east Columbus and central Columbus for two reasons. First, east Columbus samples were collected along Big Walnut Creek and Alum Creek (which flows into Big Walnut Creek), while central Columbus samples were collected along the Scioto River and the Olentangy River (which flows into the Scioto). Big Walnut Creek merges with the Scioto multiple miles downstream of where any samples were collected (Figure 1B). Second, genetic data from sequence-related amplified polymorphism (SRAP) genotyping revealed evidence that sampling sites in east Columbus differ genetically from central Columbus sites (Mattingly et al., manuscript in preparation).

I used a linear mixed effects model to investigate if the region of collection had a statistically significant effect on trait values. I dropped the Louisville population from this analysis due to low sample size (n=6 for leaf traits, n=2 for floral traits). I formulated the model as shown below:

mixed_effects_model <- lmer(some trait ~ region of collection + number of tubers +
day of year of collection + (1|GPS site), data = (data with Louisville removed), REML = TRUE)

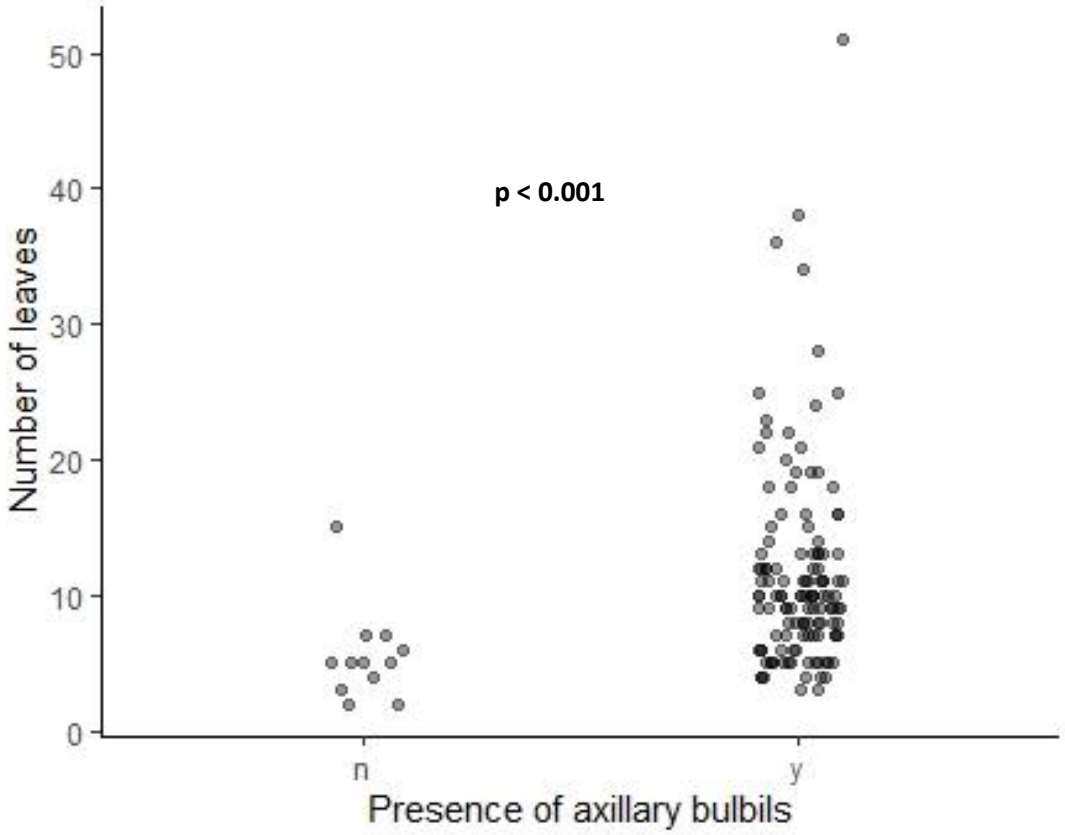
In addition to region of collection, I added two possible covariates to the model: *number of tubers at planting* and *day of year of collection*. I included *number of tubers at planting* to model trait variation based on overall plant size. I included *day of year of collection* to model any differences in traits driven by variable phenological stage. I included a random effect term, *GPS site*, to account for different site-level trait means. I ran the modeling with the lme4 package in R (Bates et. al. 2015). To test the null hypothesis that trait values do not differ by region of collection, I used a Type III Wald F test with Kenward-Roger degrees of freedom, implemented with the car package in R (Weisberg 2019). To find significance levels for pairwise comparisons between regions of collection, I used the Tukey method for comparing families of estimates, with Kenward-Roger degrees of freedom, implemented with the lsmeans package in R (Length 2016).

I compared mean trait values for each region of collection to the trait values delineated in the taxonomic key written by Sell (1994). I also compared mean trait values with the results of Post et. al (2009).

Results

Only 12 of 129 plants did not produce bulbils in the leaf axils. These 12 absences are scattered between populations: two plants collected from different sites in Cleveland, one plant from east Columbus, and nine plants from the Olentangy River in central Columbus did not produce axillary bulbils. All plants from Cincinnati and Louisville produced axillary bulbils. At least one plant collected from each of the 43 sites produced axillary bulbils. Plants with more tubers at the time of planting are more likely to produce axillary bulbils ($p=0.014$). Plants with more leaves during leaf data collection are also more likely to produce axillary bulbils ($p<0.001$, Figure 3).

Figure 3. Relationship between the presence of bulbils in leaf axils with the number of leaves during leaf trait measurement.



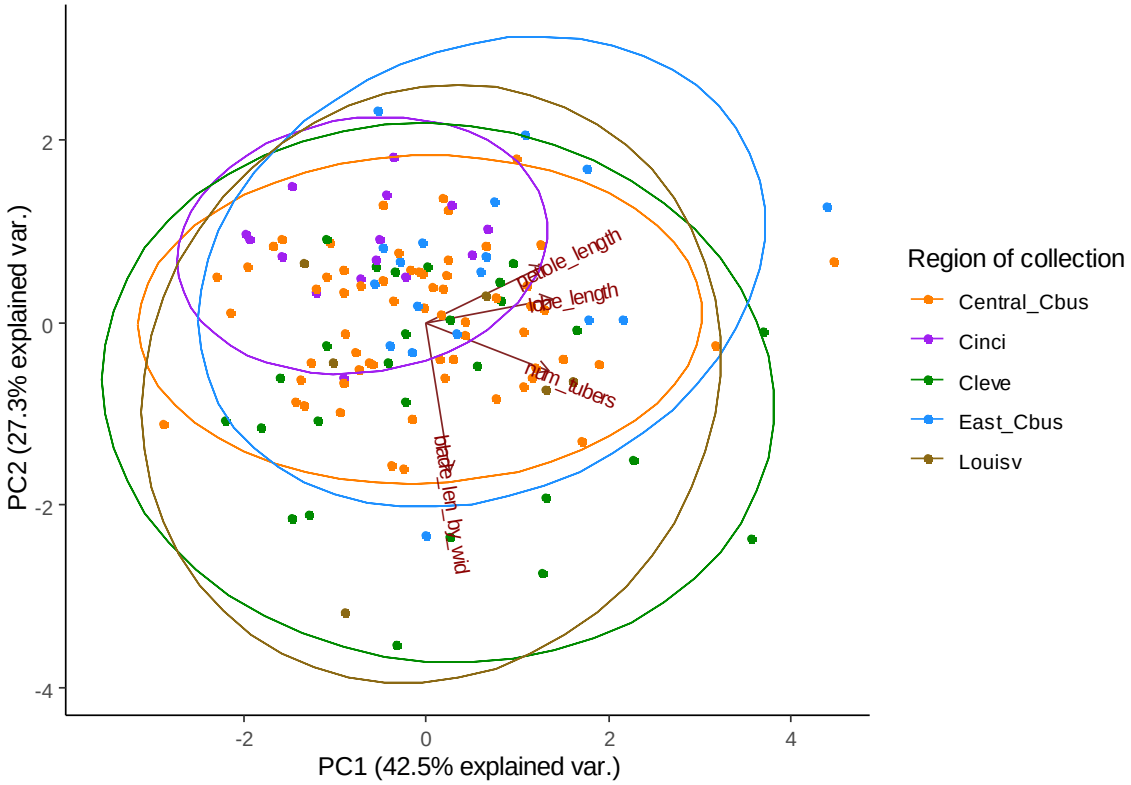
A total of 59 out of 129 sample pots produced at least one flower. Some individual plants produced up to four flowers, but most plants produced zero or one flower. None of the plants collected from Cincinnati flowered. The number of flowers a plant produces is positively correlated with the number of leaves (data not shown). After the petals of a flower fell off, between zero and 10 of the carpels in the carpel whorl began to expand in size over the course of multiple days. This indicated that a subset of the achenes in the carpel whorl were likely ripening into mature achenes. The mean number of expanded achenes per flower was 2.02 achenes. Plants from Louisville, both regions in Columbus, and Cleveland all produced expanded achenes. Of the 31 GPS sites which produced at least one flower, all 31 produced at least one expanded achene.

The first two principal component axes of the leaf trait PCA explain 70% of the variance observed (Table 2). The second PC axis is associated with the ratio of blade length to width, while the first axis is associated with the other three traits (Table 2). The leaf trait PCA shows no clear separation by region of collection in multivariate space (Figure 4), except that Cincinnati plants have a smaller leaf length to width ratio.

Table 2. Loadings for principal component axes of the leaf data PCA.

	PC1 (43% of variance)	**PC2 (27% of variance)**	**PC3 (16% of variance)**	**PC4 (15% of variance)**
number of tubers	0.58	-0.29	0.27	-0.72
blade length divided by width	0.11	-0.89	-0.30	0.34
lobe length	0.60	0.14	0.50	0.61
petiole length	0.55	0.33	-0.77	0.02

Figure 4. Biplot for the first two PC axes of the leaf data PCA. Color indicates region of collection. Ellipses are 95% bivariate normal ellipses. The variable blade_len_by_wid is a composite variable of the ratio of blade length to blade width. Sample size = 129.



The first two PC axes of the leaf+floral trait PCA explain 56% of the variance (Table 3). The second PC axis is associated with the blade length to width ratio, while the first axis is associated with all the other traits. The leaf+floral trait PCA also does not reveal separation by region in multivariate space (Figure 5).

Table 3. Loadings for the first four principal component axes of the PCA incorporating floral traits. The first four axes together explained 79% of the variance.

	PC1 (42% of variance)	**PC2 (14% of variance)**	**PC3 (13% of variance)**	**PC4 (10% of variance)**
sepal length	0.28	0.30	-0.36	0.76
petal length	0.48	0.05	-0.21	0.03
petal width	0.43	0.09	-0.23	-0.45
carpel whorl width	0.41	-0.24	-0.37	-0.26
blade length divided by blade width	-0.02	0.87	0.13	-0.22
lobe length	0.32	-0.21	0.44	0.08
petiole length	0.34	-0.08	0.51	0.27
number of tubers	0.36	0.17	0.40	-0.15

Figure 5. Biplot for the first two PC axes of the PCA incorporating floral traits. Color indicates region of collection. Ellipses are 95% bivariate normal ellipses. Sample size = 59. None of the plants from Cincinnati flowered. Sample size for Louisville was 2, so no 95% normal ellipse is drawn.

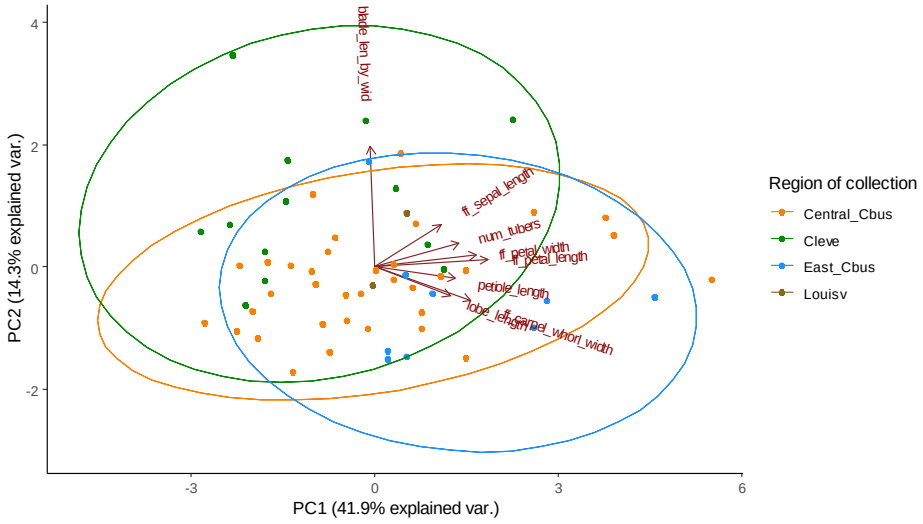
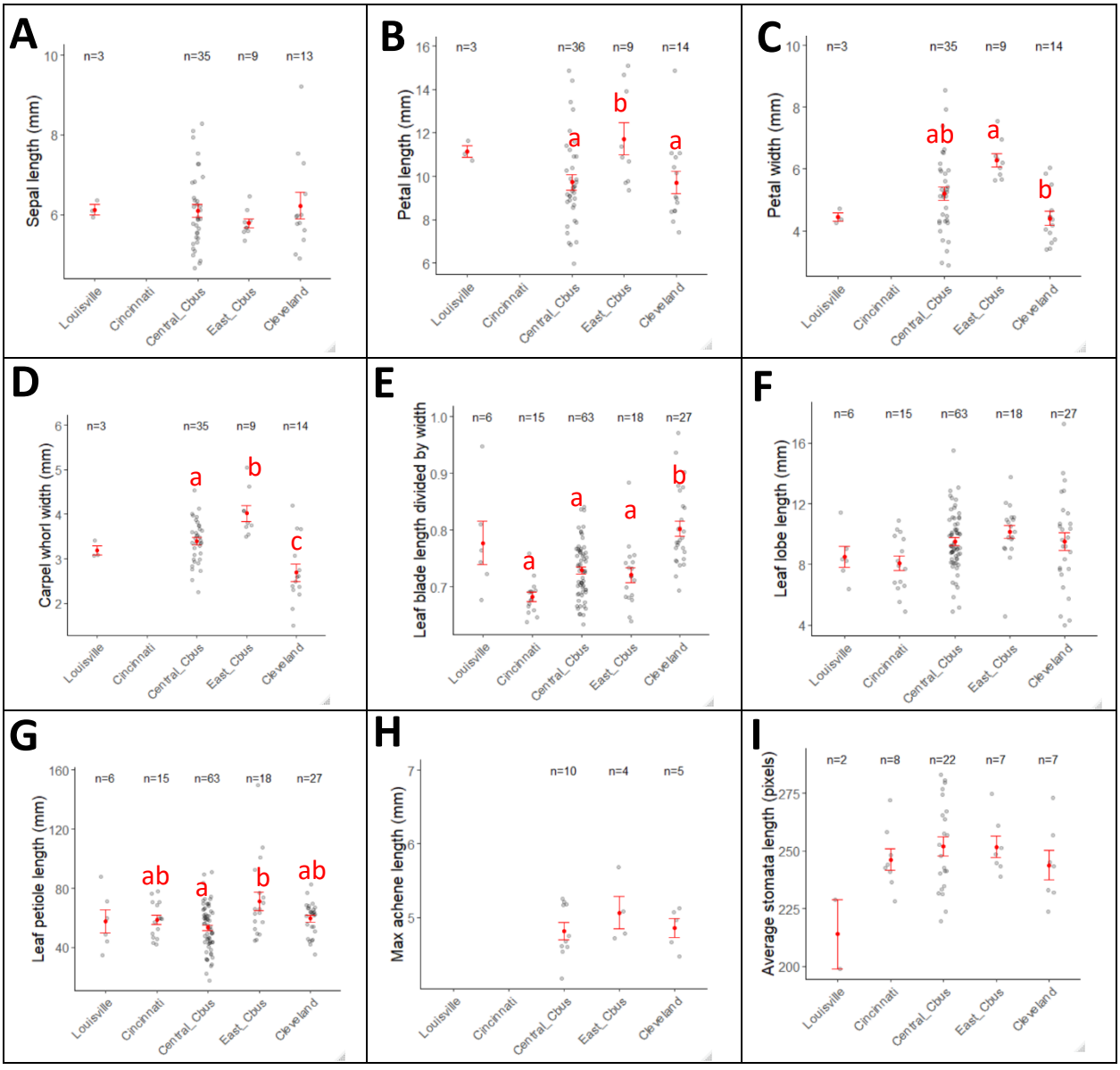


Table 4 shows the results of Type III Wald F Tests run on each linear mixed-effects model. None of the ten traits I examined vary significantly by the day of year they were collected (Table 4). However, plants with more tubers have longer petals, bigger carpel whorls, longer leaf blade length to width ratios, longer leaf blade lobes, and longer leaf petioles (Table 4). After accounting for the number of tubers, plants from east Columbus have longer petals than plants from central Columbus or Cleveland (Table 4 and Figure 6B). Cleveland plants have narrower petals than east Columbus plants (Table 4 and Figure 6C). Cleveland plants have smaller carpel whorls than central Columbus and central Columbus plants have smaller carpel whorls than east Columbus (Table 4 and Figure 6D). Leaves from Cleveland have a higher blade length to width ratio than Cincinnati, east Columbus, or central Columbus (Table 4 and Figure 6E). Leaves from east Columbus have longer petioles than those from central Columbus (Table 4 and Figure 6G).

Table 4. The p-values generated by Type III Wald's F Tests. Any p-value below $\alpha = 0.05$ is bolded. Any p-value between 0.05 and 0.10 is italicized. Note: the model for average_stomata_length_pixels was a singular fit.

Trait name	**Figure number**	**Region of collection p-value**	**Number of tubers p-value**	**Day of year of collection p-value**
sepal length	A	0.522	*0.090*	0.771
petal length	B	**0.018**	**<0.001**	0.927
petal width	C	**0.006**	*0.059*	0.789
carpel whorl width	D	**<0.001**	**0.004**	0.655
blade length divided by width	E	**<0.001**	**0.020**	0.484
lobe length	F	0.695	**<0.001**	0.866
petiole length	G	**0.018**	**<0.001**	0.569
maximum achene length	H	0.558	0.198	0.593
average stomata length	I	0.639	0.327	0.784

Figure 6. Trait values by region of collection. Error bars represent the mean ± standard error. Points are jittered along the x-axis to reveal otherwise overlapping data. Group sample sizes are indicated with n. For the traits that significantly vary by region of collection, letters above error bars represent pairwise Tukey significance (at α=0.05) from the mixed effects model, which accounts for number of tubers at planting and day of year of collection. Louisville was omitted from the mixed effects model due to low sample size.



To identify subspecies, I considered only the subspecies which produce axillary bulbils (*bulbifer* and *ficariiformis*). Trait means for leaf blade width, leaf petiole length, and flower petal length support a designation of subspecies *bulbifer* (Table 5). In contrast, the mean achene length supports subspecies

ficariiformis (Table 5). Interestingly, the mean petal width for plants from Cleveland was small enough to support subspecies *bulbifer*, while the mean petal width for plants from both Columbus populations supported subspecies *ficariiformis* (Table 5).

Table 5. Comparison of the mean trait values found in this study with the values described in Sell (1994) and Post et. al. (2009). The two subspecies considered here, *bulbifer* and *ficariiformis*, are the only subspecies which produce axillary bulbils. The measured traits are color-coded to indicate the subspecies they are closest to.

Trait	Sell 1994 – *ficariiformis* max. dimensions (or typical range)	Post et. al. 2009 – *ficariiformis* mean dimensions	Sell 1994 – *bulbifer* max. dimensions (or typical range)	Post et. al. 2009 – *bulbifer* mean dimensions	This study – mean Cincinnati	This study – mean central Columbus	This study – mean east Columbus	This study – mean Cleveland
leaf length including lobe	7 cm	2.97 cm	4 cm	2.18 cm	2.6 cm	3.1 cm	3.2 cm	3.4 cm
leaf width	7 cm	3.80 cm	4 cm	2.83 cm	2.6 cm	2.9 cm	3.0 cm	3.0 cm
petiole length	28 cm	14.75 cm	15 cm	10.41 cm	6 cm	5.5 cm	7 cm	6 cm
petal length	17-26 mm	13.91 mm	6-11 mm	10.23 mm	none flowered	10 mm	11.5 mm	10 mm
petal width	4-12 mm	5.75 mm	2-5 mm	3.57 mm	none flowered	5.2 mm	6.2 mm	4.2 mm
achene length	4-5 mm	4.70 mm	not listed	4.35 mm	none flowered	4.8 mm	5 mm	4.8 mm
achene width	2.5-3.5 mm	3.5 mm	not listed	2.95 mm	none flowered	not measured	not measured	not measured

Discussion

We documented widespread axillary bulbil production in all four cities of collection. We discovered a strong dependence of axillary bulbil production on below- and above-ground biomass (through the proxies of number of tubers at planting and number of leaves, respectively). Since *R. ficaria* is a perennial and uses the belowground tubers for energy storage, it makes sense that larger plants with more resources would have the ability to invest in clonal dispersal.

A surprising result from our study was that plants which flowered typically produced one or more expanded, presumably ripened achenes. These ripened achenes raise the possibility that sexual

reproduction is possible for *R. ficaria* populations in Ohio and Kentucky. This finding is particularly interesting given that Cleveland populations of *R. ficaria* have not been observed to produce expanded achenes (Emily Rauschert, personal communication), but Columbus populations do produce expanded achenes in the wild (personal observation). One explanation is that the simultaneous blooming of flowers from Cleveland and from Columbus or Louisville in proximity, coupled with the presence of insect pollinators at the study site, could have led to cross pollination during this experiment. Given that Columbus populations of *R. ficaria* differ genetically from Cleveland populations, this finding provides preliminary evidence that increases in genetic diversity might worsen invasion severity. However, there are multiple caveats. For one, we did not test the viability of the expanded achenes. Multiple stratifications might be needed to break the dormancy of ripened achenes (Axtell et. al. 2009). Bulbiferous *R. ficaria* was shown to often have disorganization in the ovary, leading to achenes which may expand and produce endosperm but are nevertheless inviable (Metcalfe 1939). Finally, since we did not control pollinations in the experiment, we cannot distinguish between achenes that were fertilized by pollen from another city, from the same city, or from the same plant or flower (selfing). It is possible for *R. ficaria* to produce viable offspring either through cross pollination or self-pollination (Marsden-Jones and Turrill 1952). Furthermore, we do not know if apomixis occurred, which has been previously documented in *R. ficaria* (Metcalfe 1939).

In addition to the results about sexual and clonal reproduction in Ohio populations of *R. ficaria*, we documented both within-and between-city morphological diversity in *R. ficaria* in Ohio and Kentucky. Cleveland populations have small flowers with narrow petals and small carpel whorls, while also having long leaf blade lengths. Eastern Columbus populations have larger flowers with larger petals and carpel whorls, as well as longer leaf petioles. Central Columbus populations are intermediate between east Columbus and Cleveland in multiple traits. Because east Columbus trait values are different from those of central Columbus, it is likely that the invasion in Columbus has multiple sources,

perhaps of different horticultural cultivars of *R. ficaria*. It is also possible that *R. ficaria* could spread from one city to another by becoming lodged in human shoes.

When attempting to identify the plants to subspecies level based on published keys, I encountered difficulties. Some traits I measured supported subspecies *bulbifer*, while others supported *ficariiformis*. There could be multiple explanations. First, the taxonomic key I used was developed in Europe and might not apply exactly to trait values measured in the United States (Sell 1994). The other source of information I used was based on herbarium specimens from North America (Post et. al. 2009), which sometimes had small sample sizes for certain traits of some subspecies. My experiment measured traits of living plants cultivated in pots. The living plants I measured in my experiment definitely did not grow to their maximum size in the wild. Given the strong dependence of floral and leaf trait values on biomass (Table 4), that fact that I did not make the subspecies determination with 'fully grown' plants might obstruct my conclusion.

There are a few limitations to this study. One of the major limitations surrounds the differences in life stage of the plants collected. During replanting of samples, plants with large numbers of belowground tubers were selected, if possible, to reduce the likelihood of death from transplanting. However, certain samples, particularly those from Cincinnati, but also some from Columbus and Cleveland, had as little as one tuber at planting. In some cases, this tuber was an axillary bulbil. In other words, in this study I am comparing plants which might be in their first, second, or even third or later year of growth. I attempted to correct for this by incorporating the number of tubers at planting as a covariate term in the linear mixed-effects model, but it is possible that the relationship is not fully captured by the linear model. Another related limitation of this study is that since plants were grown from tubers instead of from seed, the previous environmental conditions at the collection site might influence trait values. For instance, a perennial plant might adjust its growth form depending on how

much flooding occurs at a site. Future work should study trait values of *R. ficaria* grown from axillary bulbils or seeds, which will minimize the effect of the environment.

In summary, we have documented within- and between-city morphological diversity in populations of *R. ficaria*. We have found that seed production might be an important factor in the success and spread of this species in Ohio. We have corroborated the importance of clonal reproduction for the spread of this species along riverways. Future work should focus on whether different genotypes of *R. ficaria* can intercross and produce viable offspring. Although the species is so widespread in Columbus, Cincinnati, and Cleveland that eradication may be impossible, certain populations could be identified and perhaps controlled to prevent or reduce seed production. From a pure-science perspective, this species provides ample research questions about the importance of sexual and asexual (clonal) reproduction in the success of biological invasions.

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